Appln. No. 09/699,224 Amdt. dated August 26, 2003 Reply to Office Action of Feb. 26, 2003

Amendments to the Specification:

Please add the following paragraph between lines 3 and 4 on page 1.

This application claims the benefit of United States provisional application 60/162,491 filed October 29, 1999, which is hereby incorporated by reference herein in its entirety.

Please replace the paragraph on page 9, lines 13-20 with the following paragraph:

Figure 8 Figures 8A-8D [[shows]] show octa-MAP1-induced IgG anti-LOS antibody responses in mice. (A) Eight mice received a dose of 50 µg of Octa-MAP1 emulsified in Freund's adjuvant on day 0 and again on day 21. (B) Four mice were immunized with purified LOS as a positive control. Mice were immunized with either Freund's adjuvant (C) or an unrelated octa-MAP control peptide (D) as negative controls.

Please replace the paragraph on page 16, line 20 to page 17 line 9 with the following paragraph:

Positive *E. coli* clones were grown overnight in IMC media containing 100 μg/ml ampicillin, at 25°C and then induced to express the peptide fusions for 6 h. *E. coli* cells were fixed with 0.5% paraformaldehyde on ice for 10 min. Aliquots of 200-μl of fixed organisms were spun at 2000 X g for 10 min. Supernatants were discarded, and pellets were resuspended in blocking buffer (IMC media containing 100 μg/ml ampicillin, 1% nonfat dry milk, 150 mM NaCl and 1% α - methyl mannoside) containing mAb 2C7. Suspensions were incubated at 37°C for 30 min. before spinning at 2000 X g for 10 min. Pellets were washed with 100 μl of washing buffer (IMC media containing 100μg/ml ampicillin and 1% α-methyl mannoside) and then resuspended in 100 μl of blocking buffer containing FITC-conjugated anti-mouse IgG (Sigma, St. Louis, MO). The mixtures were incubated at 37°C for 30 min before spinning at 2000 X g for 10 min. Supernatants were removed, and pellets washed in 100 μl of washing buffer before resuspension in 1 ml of PBS. The suspensions were analyzed on a FACS using CellQuest software CELLQUEST Flow Cytometry Software (Becton Dickinson, Franklin Lakes NJ). A negative clone that did not bind mAb 2C7 was used as a control.

Please replace the paragraph on page 18, lines 13-22 with the following paragraph:

A synthetic peptide (PEP1; IPVLDENGLFAP [SEQ ID NO:1]) whose sequence corresponds to the consensus sequence "DE_GLF" and includes two cysteine flanking regions (CGP- and -GPC residues at the [[N]]- and C- terminus, respectively) was synthesized (Boston Biomolecules, MA) to assess specific binding to 2C7 mAb by inhibition ELISA and to determine whether peptide mimics characterized as thioredoxinfusion proteins would retain the antigenicity independent of the fusion context [SEQ ID NO:10].

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Please replace the paragraph on page 18, line 31 to page 19 line 14 with the following paragraph:

Peptides were diluted in blocking buffer (1% ovalbumin, 0.05% tween-20 TWEEN-20TM (polysorbate 20), 0.5 M NaCl in PBS) to produce mixtures of varying concentrations (0.1, 0.5 and 1 mg/ml). 50 μl-aliquots from each of the concentrations were incubated with 50 μl of mAb 2C7 (stock concentration 2 μg/ml diluted in blocking buffer) at 37°C for 1 h, then 100 μl of the mixtures were loaded into microtiter plate wells coated with purified LOS prepared from strain 15253 (80 μg/ml). The wells were incubated at 37°C for 1 h, then washed. After the wells were washed, bound mAb 2C7 was detected with anti-mouse IgG conjugated to alkaline phosphatase. Purified LOS prepared from gonococcal strain 15253 was used as a positive control. A non-reactive 15-mer peptide sequence generated by the above described random peptide library system was used as a negative control peptide [SEQ ID NO:9].

Please replace the paragraph on page 20, line 23 to page 21, line 10 with the following paragraph:

Solid phase ELISA was performed to assess the binding of mAb 2C7 to multiple antigen peptides. For direct ELISA, Immulon 1 plates were coated overnight with multiple antigen peptides (1 μg/well) and reacted with varying concentration of mAb 2C7. For inhibition ELISA, plates were coated with purified LOS prepared from *N. gonorrhoeae* strain 15253 (80 μg/ml) at 37°C for 3 h. Peptides (linear or MAPs) were diluted in blocking buffer (1% ovalbumin, 0.05% tween-20 TWEEN-20TM (polysorbate 20),0.5 M NaCl in PBS) to produce mixtures of varying concentrations. 50 μl-aliquots from each concentration were incubated with 50 μl of mAb 2C7 (stock concentration 0.4 μg/ml diluted in blocking buffer) at 37°C for 1 h, then 100 μl of mixtures were loaded into microtiter plate wells. The wells were incubated at 37°C for 1 h, then washed. After the wells were washed, bound mAb 2C7 was detected with anti-mouse IgG conjugated to alkaline phosphatase. Purified LOS prepared from gonococcal strain 15253 was used as a positive control in inhibition ELISA.

Please replace the paragraph on page 21, line 23 with the following paragraph:

Immunization with octa-MAP1 induces an IgG anti-LOS antibody response in mice, as shown in Figure 8 Figures 8A-8D. The response profile seen in Figure 8(A), in which there is no significant IgG anti-LOS response until the boost at week 3, indicates that the Octa-MAP1 elicited a T-cell dependent immune response in the responding mice. These results demonstrate the promise of a peptide mimic, such as Octa-MAP1, for immunizing humans against N. gonorrhoeae infection.

Please replace the paragraph on page 21, line 32 with the following paragraph:

In Figure 8(A), eight mice received a dose of 50 µg of Octa-MAP1 emulsified in Freund's adjuvant on day 0 and again on day 21. Octa-MAP1, which mimics the 2C7 oligosaccharide epitope, induced IgG anti-LOS antibody in three of the eight mice. IgG

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anti-LOS responses in these three mice rose significantly after the first boost at week 3, peaked at week 7 (the next time measured) and decreased thereafter. Figure 8(B) shows the positive control experiment in which four mice were immunized with purified LOS. In these mice, IgG anti-LOS titers increased minimally after the first immunization and rose after boosting. All mice in the LOS group showed an anti-LOS antibody response. Four mice immunized with either Freund's adjuvant (C) or an unrelated octa-MAP control peptide (D), both negative controls, elicited weak or no IgG anti-LOS responses. The mean IgG anti-LOS antibody responses from all immunized mice (from the experiments depicted in Figure 8 Figures 8A-8D) are shown in Figure 9 (mean ± SE, including animals that exhibited no response).

Please replace the paragraph on page 22, line 18 with the following paragraph:

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IgG anti-LOS antibody responses for the responder mice only (from the experiments depicted in Figure 8 Figures 8A-8D) are shown in Figure 10. Antibody response is defined as IgG anti-LOS (mean \pm SE) greater than 0.4 mg/ml (4 fold above baseline IgG anti-LOS levels). At 7 and 10 weeks after primary immunization, responder mice immunized with Octa-MAP1 elicited IgG anti-LOS antibody levels higher (p < 0.001) than antibody levels elicited by negative control antigens (Freund's adjuvant alone or unrelated octa-MAP control peptide).

Please replace the paragraph on page 22, line 28 with the following paragraph:



IgM anti-LOS antibody responses for responder mice only (from the experiments depicted in Figure 8 Figures 8A-8D) are shown in Figure 11. Mice immunized with Octa-MAP1 that had elicited IgG anti-LOS responses failed to respond with IgM anti-LOS levels higher than mice immunized with negative control antigens. Immunization with LOS (positive control) elicited IgM anti-LOS antibody levels higher than animals immunized with either Octa-MAP1 or negative control antigens (Freund's adjuvant alone or unrelated octa-MAP control peptide).